

US Appl. No: 10/538,405
Response to Restriction Requirement

RECEIVED
CENTRAL FAX CENTER

SEP 21 2007

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions and listings of claims in the application:

1. (Previously presented) A method for producing a protein, the method comprising the steps of:
 - (a) providing a nucleic acid sequence coding for the protein wherein the nucleic acid sequence coding for the protein comprises a translation start codon;
 - (b) inserting a heterologous nucleic acid sequence on the 3' side of the translation start codon in the correct reading frame, wherein said heterologous nucleic acid sequence forms a stem-loop structure on the 3' side of the translation start codon 6-30 nucleotides from the 3' side of the start codon;
 - (c) providing an expression system for the protein;
 - (d) introducing the nucleic acid sequences combined in step (b) into the expression system; and
 - (e) forming the stem-loop structure wherein the length of the stem is in the range of 4-12 nucleotides.
2. (Previously presented) The method as claimed in claim 1 further comprising the step of isolating the protein.
3. (Previously presented) The method as claimed in claim 1 wherein the heterologous nucleic acid sequence has a length of up to 201 nucleotides.
4. (Previously presented) The method as claimed in claim 3 wherein the heterologous nucleic acid sequence has a length of up to 45 nucleotides.
5. (Previously presented) The method as claimed in claim 1 wherein the stem-loop structure is formed 12-21 nucleotides from the 3' side of the start codon.
6. (Previously presented) The method as claimed in claim 1 wherein the region of the heterologous nucleic acid sequence that is on the 5' side of the stem-loop

US Appl. No: 10/538,405
Response to Restriction Requirement

structure does not form a secondary structure with the 5' untranslated region of the nucleic acid sequence coding for the protein.

7. (Previously presented) The method as claimed in claim 1 wherein the region of the heterologous nucleic acid sequence that is on the 5' side of the stem-loop structure and on the 3' side of the start codon has a GC content of less than 50 %.

8. (Previously presented) The method as claimed in claim 1 wherein an *in vitro* expression system is used.

9. (Previously presented) The method as claimed in claim 8 wherein the *in vitro* expression system is a prokaryotic *in vitro* expression system.

10. (Previously presented) The method as claimed in claim 9 wherein the prokaryotic *in vitro* expression system comprises a lysate of *Escherichia coli* or of *Bacillus subtilis*.

11. (Previously presented) The method as claimed in claim 8 wherein the *in vitro* expression system is a eukaryotic *in vitro* expression system.

12. (Previously presented) The method as claimed in claim 11 wherein the eukaryotic *in vitro* expression system comprises a lysate selected from the group consisting of a lysate of mammalian cells, reticulocytes, human tumour cell lines, hamster cell lines, other vertebrate cells, oocytes, eggs of fish, eggs of amphibia, insect cell lines, yeast cells, algal cells, and extracts of plant seedlings.

13. (Previously presented) The method as claimed in claim 1 wherein the expression system is a prokaryotic *in vivo* expression system.

14. (Cancelled)

15. (Previously presented) The method as claimed in claim 13 wherein the prokaryotic expression system comprises an *E. coli* cell or a *Bacillus subtilis* cell.

US Appl. No: 10/538,405
Response to Restriction Requirement

16. (Previously presented) The method as claimed in claim 1 wherein the expression system comprises a eukaryotic host cell.

17. (Previously presented) The method as claimed in claim 16 wherein the eukaryotic host cell is selected from the group consisting of a yeast cell, an insect cell, an amphibian cell, a fish cell, a bird cell, a mammalian cell, and a vertebrate cell.

18. (Previously presented) The method as claimed in claim 16 wherein the expression system is a non-human eukaryotic host organism.

19. (Previously presented) The method as claimed in claim 1 wherein the nucleic acid sequence coding for the protein is provided by a method selected from the group consisting of cloning, recombination and amplification.

20. (Previously presented) The method as claimed in claim 19 wherein the nucleic acid sequence coding for the protein is provided by a two-step polymerase chain reaction.

21. (Previously presented) The method as claimed in claim 1 wherein the nucleic acid sequence coding for the protein or the heterologous nucleic acid sequence comprises a codon adapted, based on codon usage, to the expression system.

22. (Previously presented) The method as claimed in claim 1 wherein the heterologous nucleic acid sequence comprises coding sequence for a purification domain or for a proteinase recognition domain.

23. (Previously presented) A composition for producing a protein, the composition comprising:

(a) a nucleic acid sequence that is heterologous to the nucleic acid sequence coding for the protein wherein the heterologous nucleic acid sequence is inserted into the protein-coding nucleic acid sequence in the correct reading frame and wherein the

US Appl. No: 10/538,405
Response to Restriction Requirement

heterologous nucleic acid sequence forms a stem-loop structure 6-30 nucleotides from the 3' side of the translation start codon; and

(b) an expression system for the protein.